

**2003080-0081  
(SK-719-Z)**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Danishefsky *et al.*  
Serial No.: not yet assigned  
Filed: April 12, 2001  
For: *Colon Cancer KH-1 and N3 Antigens*

Examiner: Ungar, S.  
Group Art Unit: 1642

BOX PATENT APPLICATION  
ASSISTANT COMMISSIONER FOR PATENTS  
WASHINGTON, DC 20231

**EXPRESS MAIL NO.: EL603009610US**

Sir:

**PRELIMINARY AMENDMENT**

Applicants respectfully request entry of the following amendments in the divisional application submitted under 37 C.F.R. § 1.53(b) herewith:

Applicants respectfully request entry of the following amendments:

**In the claims:**

Please cancel claims 75-84 and 100, which claims were elected for prosecution in the parent case 09/042,280.

**In the specification:**

Please amend the paragraph on page 1, lines 6-12, as follows:

This application is [based on] a divisional application filed under 37 C.F.R. § 1.53(b) of application number 09/042,280, filed January 13, 1998, which further claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial No. 60/034,950, filed January 13, 1997, and the entire contents of [which] each of these applications are hereby incorporated by reference into this application. This invention was made with government support under grants CA-

28824-18, GM-15240-02, GM-16291-01, HL-25848-14 and AI-16943 from the National Institutes of Health. Additionally, the present invention was supported in part by a fellowship from the United States Army to Hyun Jin Kim (DAMD 17-97-1-7119). Accordingly, the U.S. Government has certain rights in the invention.

Please amend pages 14-16 as follows:

On page 14, line 11, please delete “Figure 2 provides” and replace with: --Figures 2(A) and 2(B) provide--

On page 14, line 25, please delete “Figure 3 provides” and replace with: --Figures 3(A) and 3(B) provide--

On page 15, line 10, please delete “Figure 5 provides” and replace with: --Figures 5(A), 5(B) and 5(C) provide--

On page 15, line 13, please delete “Figure 6 provides” and replace with: --Figures 6(A), 6(B) and 6(C) provide--

On page 15, line 16, please delete “Figure 7 provides” and replace with: --Figures 7(A) and 7(B) provide--

On page 15, line 22, please delete “Figure 9 provides” and replace with: --Figures 9(A) and 9(B) provide--

On page 15, line 25, please delete “Figure 10 provides” and replace with --Figures 10(A) and 10(B) provide--

On page 15, line 28, please delete "Figure 11 provides" and replace with --Figures 11(A) and 11(B) provide--

On page 15, line 31, please delete “Figure 12 illustrates” and replace with: --Figures 12(A) and 12(B) illustrate--

On page 16, line 5, please delete “Figure 13 illustrates” and replace with: --Figures 13(A) and 13(B) illustrate--

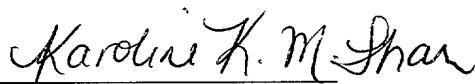
## R E M A R K S

Applicants respectfully request entrance of the amendments as detailed above, in the divisional application filed herewith under 37 C.F.R. § 1.53(b). Applicants respectfully submit that no new matter is presented with these amendments. Rather, the original specification, as filed on January 13, 1998, has been provided for filing under 37 C.F.R. §1.53 (b), and Applicants respectfully submit that this preliminary amendment is requested to reflect cancellation of non-elected claims, and to correct formal matters in the specification (e.g., addition of a statement of divisional application status (with incorporation by reference), addition of a statement regarding government support, and ensuring consistency between the specification and formal drawings). Applicants have provided replacement pages 14-16 reflecting the amendments made, as described in more detail above, and additionally have provided a replacement paragraph for the paragraph found on page 1, lines 6-12.

As detailed above, Applicants have requested cancellation of claims 74-82 and 100, and respectfully request examination of claims 1-73, 83-99 and 101-107 on the merits. Applicants explicitly reserve the right, however, to present additional claims directed to subject matter in cancelled claims 74-82 and 100, which subject matter is additionally found throughout the specification, in future continuation or divisional applications.

Applicants would like to thank the Examiner in advance for review of this request. If it is believed that a telephone conversation would expedite matters, the Examiner is invited to contact the undersigned at (617) 248-5216. Although it is believed that there is no fee associated with this amendment, if Applicants are mistaken, please charge any fees to our Deposit Account No.: 03-1721.

Respectfully Submitted,

  
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**EXPRESS MAIL NO.: EL603009610US**

Sir:

**REPLACEMENT PARAGRAPH AND PAGES 14-16**

Replacement paragraph for the paragraph found at page 1, lines 6-12:

This application is a divisional application filed under 37 C.F.R. § 1.53(b) of application number 09/042,280, filed January 13, 1998, which further claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial No. 60/034,950, filed January 13, 1997, and the entire contents of each of these applications are hereby incorporated by reference into this application. This invention was made with government support under grants CA-28824-18, GM-15240-02, GM-16291-01, HL-25848-14 and AI-16943 from the National Institutes of Health. Additionally, the present invention was supported in part by a fellowship from the United States Army to Hyun Jin Kim (DAMD 17-97-1-7119). Accordingly, the U.S. Government has certain rights in the invention.

### Brief Description of the Drawings

**Figure 1** shows the structure of the cell surface antigen KH-1 ceramide and its  
5 bioconjugateable O-allyl ether form.

**Figures 2(A) and 2(B)** provide synthetic Scheme 1. Reagents: (a) (i) 3,3-dimethyldioxirane, CH<sub>2</sub>Cl<sub>2</sub>; (ii) **4** or **5**, ZnCl<sub>2</sub> THF 65% for **6** 55% for **7**; (b) (i) TESOTf, Et<sub>3</sub>N, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 92%, (ii) I(coll)<sub>2</sub>ClO<sub>4</sub>, PhSO<sub>2</sub>NH<sub>2</sub>, 4 Å molecular  
10 sieves, CH<sub>2</sub>Cl<sub>2</sub>, > 90%; (iii) LHMDS, EtSH, DMF > 90%, (iii) LHMDS, EtSH, DMF  
(iv) Ac<sub>2</sub>O, Et<sub>3</sub>N, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 85%; (d) K<sub>2</sub>CO<sub>3</sub>, MeOH 80%; (e) (i) MeOTf, di-t-butylpyridine, Et<sub>2</sub>O:CH<sub>2</sub>Cl<sub>2</sub> (2:1), 4 Å MS (55%), (ii) K<sub>2</sub>CO<sub>3</sub>, MeOH (85%); (f) (i) MeOTf, di-t-butylpyridine, Et<sub>2</sub>O:CH<sub>2</sub>Cl<sub>2</sub> (2:1), 4 Å MS (60%); (ii) Ac<sub>2</sub>O, Py, DMAP,  
15 CH<sub>2</sub>Cl<sub>2</sub> (95%); (g) TBAF:AcOH (93%).

15

**Figures 3(A) and 3(B)** provide synthetic Scheme 2. Reagents (a) **14**, Sn(OTf)<sub>2</sub>, Tol:THF (10:1), 4 Å MS (60%); (b) (i) 3,3-dimethyldioxirane, CH<sub>2</sub>Cl<sub>2</sub>; (ii) EtSH, CH<sub>2</sub>Cl<sub>2</sub>, H<sup>+</sup> (cat); (iii) Ac<sub>2</sub>O, Py, CH<sub>2</sub>Cl<sub>2</sub> 60% (3 steps) (c) **17**, MeOTf, Et<sub>2</sub>O:CH<sub>2</sub>Cl<sub>2</sub>  
15 (2:1), 4 Å MS (55%); (d) (i) Lindlar's catalyst, H<sub>2</sub>, palmitic anhydride, EtOAc, 85% (ii) Na, NH<sub>3</sub>, THF; (MeOH quench); (iii) Ac<sub>2</sub>O, Et<sub>2</sub>N, DMAP, CH<sub>2</sub>Cl<sub>2</sub> (iv) MeONa, MeOH,  
20 70% (3 steps); (e) (i) Na, NH<sub>3</sub>, THF; (MeOH quench); (ii) Ac<sub>2</sub>O, Et<sub>3</sub>N, DMAP, CH<sub>2</sub>Cl<sub>2</sub>;  
(iii) 3,3-dimethyldioxirane, CH<sub>2</sub>Cl<sub>2</sub>; (iv) Allyl Alcohol (v) MeONa, MeOH, 60%.

**Figure 4** provides a synthetic strategy for N3 antigen.

5

**Figures 5(A), 5(B) and 5(C)** provide a synthetic strategy for the Le x donor portion.

**Figures 6(A), 6(B) and 6(C)** provide a synthetic strategy for the Le a donor portion.

10 **Figures 7(A) and 7(B)** provide a synthetic strategy for the N3 acceptor portion.

**Figure 8** provides a 2 + 2 coupling for the major N3 antigen.

**Figures 9(A) and 9(B)** provide a 2 + 4 and 1+ 1 coupling for the N3 antigen.

15

**Figures 10(A) and 10( B)** provide a pathway for deprotection of the major N3 epitope.

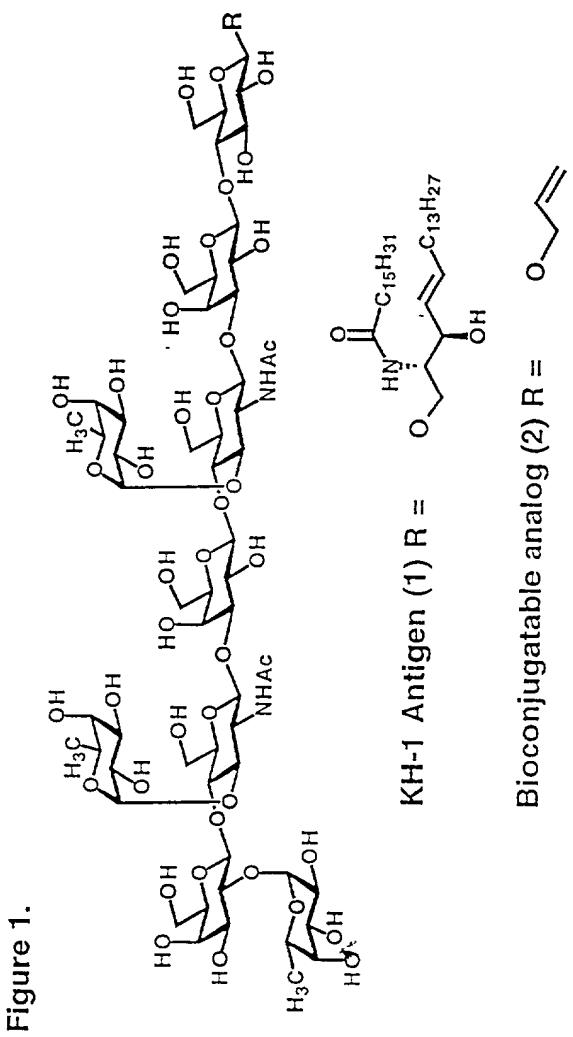
**Figures 11(A) and 11(B)** provide a synthetic strategy for the KH-1 tetrasaccharide and hexasaccharide intermediates.

20

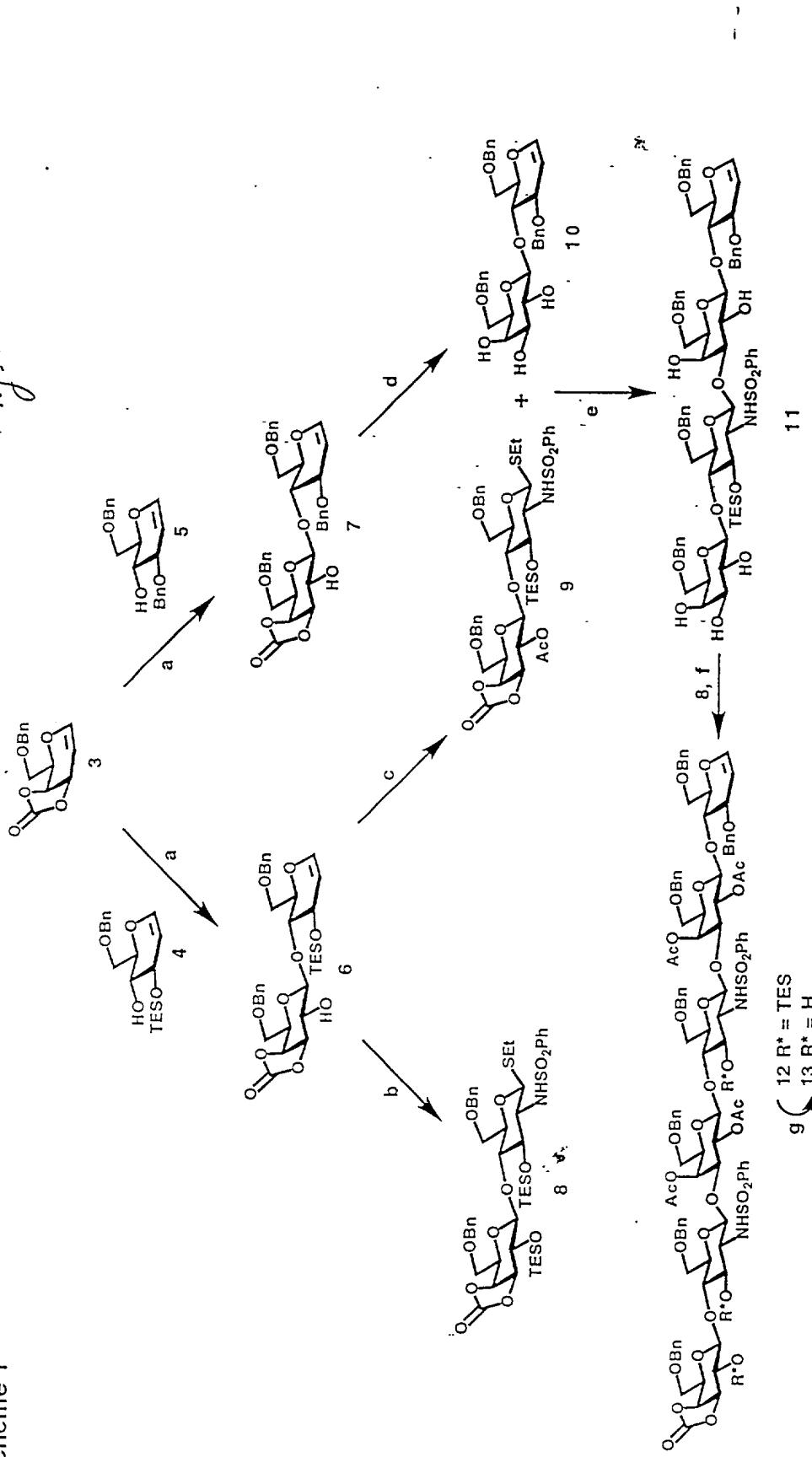
**Figures 12(A) and 12(B)** illustrate the direct coupling of KH-1 to KLH.

**Figures 13(A) and 13(B)** illustrate the coupling of KH-1 to KLH via a M<sub>2</sub> cross-linker.

JC929 U.S. PRO  
09/033327  
04/12/01



Scheme 1a



<sup>a</sup> Reagents: (a) (i) 3,3-dimethylidioxirane,  $\text{CH}_2\text{Cl}_2$ ; (ii) 4 or 5,  $\text{ZnCl}_2$ , THF 65% for 6 & 55% for 7; (b) (i) TESOTf,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , 92%, (ii)  $(\text{coll})_2\text{ClO}_4$ ,  $\text{PhSO}_2\text{NH}_2$ , 4 Å molecular sieves,  $\text{CH}_2\text{Cl}_2$ , > 90%; (iii) LHMDS, EtSH, DMF > 90%; (c) (i)  $\text{Ac}_2\text{O}$ ,  $\text{Et}_3\text{N}$ , DMAP,  $\text{CH}_2\text{Cl}_2$ , 95%; (ii)  $(\text{coll})_2\text{ClO}_4$ ,  $\text{PhSO}_2\text{NH}_2$ , 4 Å molecular sieves,  $\text{CH}_2\text{Cl}_2$ , > 90%; (iii) LHMDS, EtSH, DMF (iv)  $\text{Ac}_2\text{O}$ ,  $\text{Et}_3\text{N}$ , DMAP,  $\text{CH}_2\text{Cl}_2$ , 85%; (d)  $\text{K}_2\text{CO}_3$ ,  $\text{MeOH}$  80%; (e) (i)  $\text{MeOTf}$ , di-*t*-butylpyridine,  $\text{Et}_2\text{O}:\text{CH}_2\text{Cl}_2$  (2:1), 4 Å MS (55%), (ii)  $\text{K}_2\text{CO}_3$ ,  $\text{MeOH}$  (85%); (f) i)  $\text{MeOTf}$ , di-*t*-butylpyridine,  $\text{Et}_2\text{O}:\text{CH}_2\text{Cl}_2$  (2:1), 4 Å MS (60%); ii)  $\text{Ac}_2\text{O}$ ,  $\text{Py}$ , DMAP,  $\text{CH}_2\text{Cl}_2$  (95%); (g)  $\text{TBAF}\cdot\text{AcOH}$  (93%).

Scheme 2<sup>a</sup>

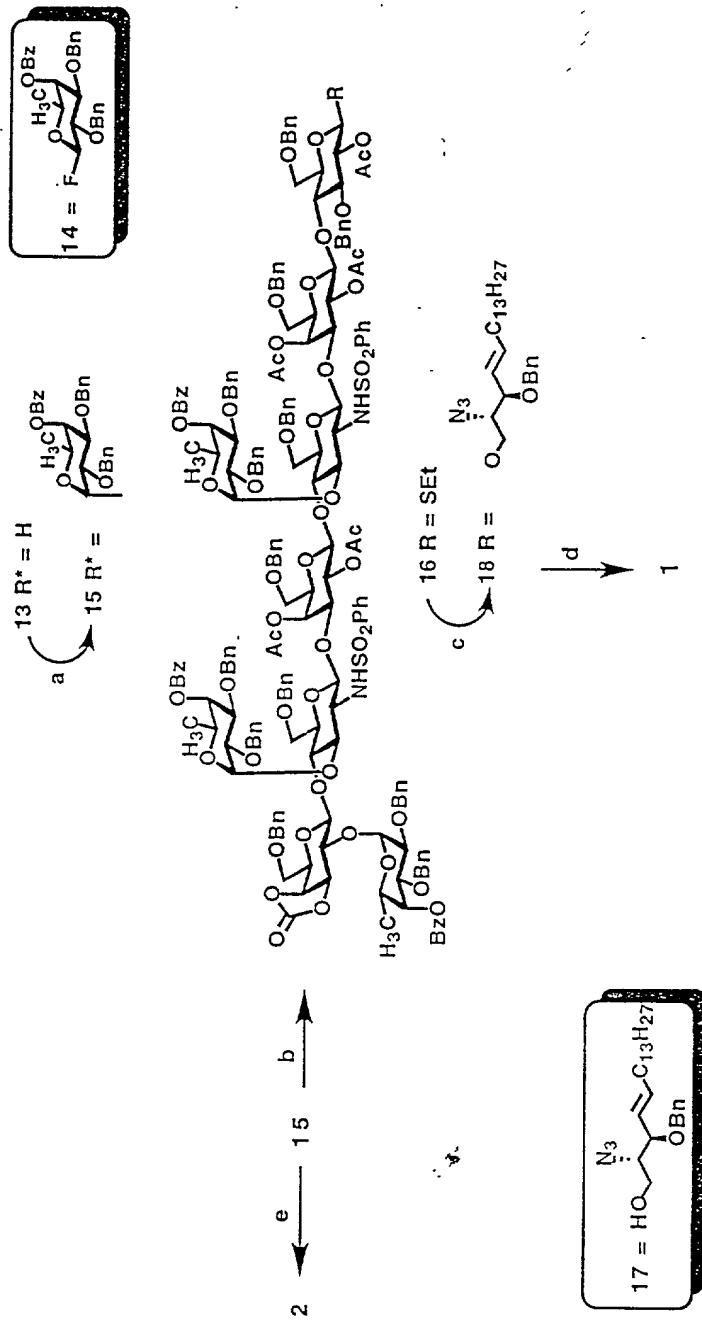
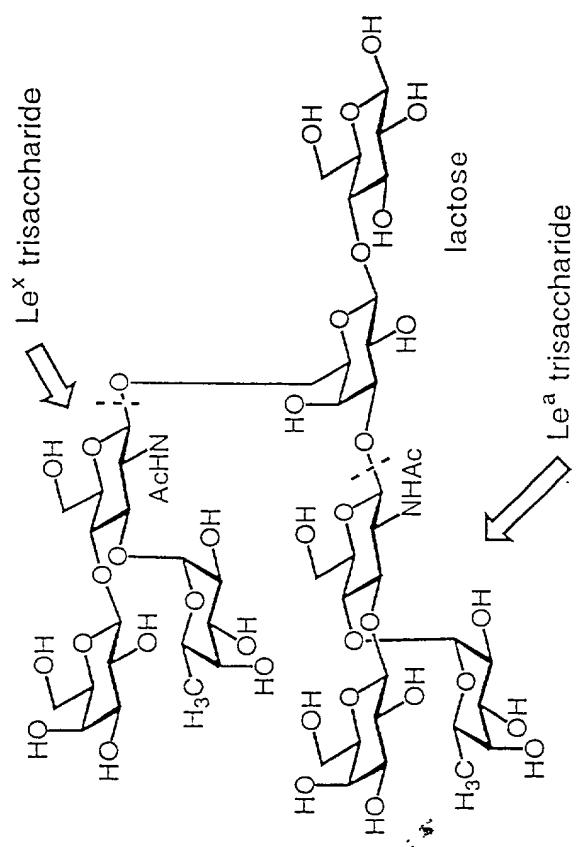


Fig. 3

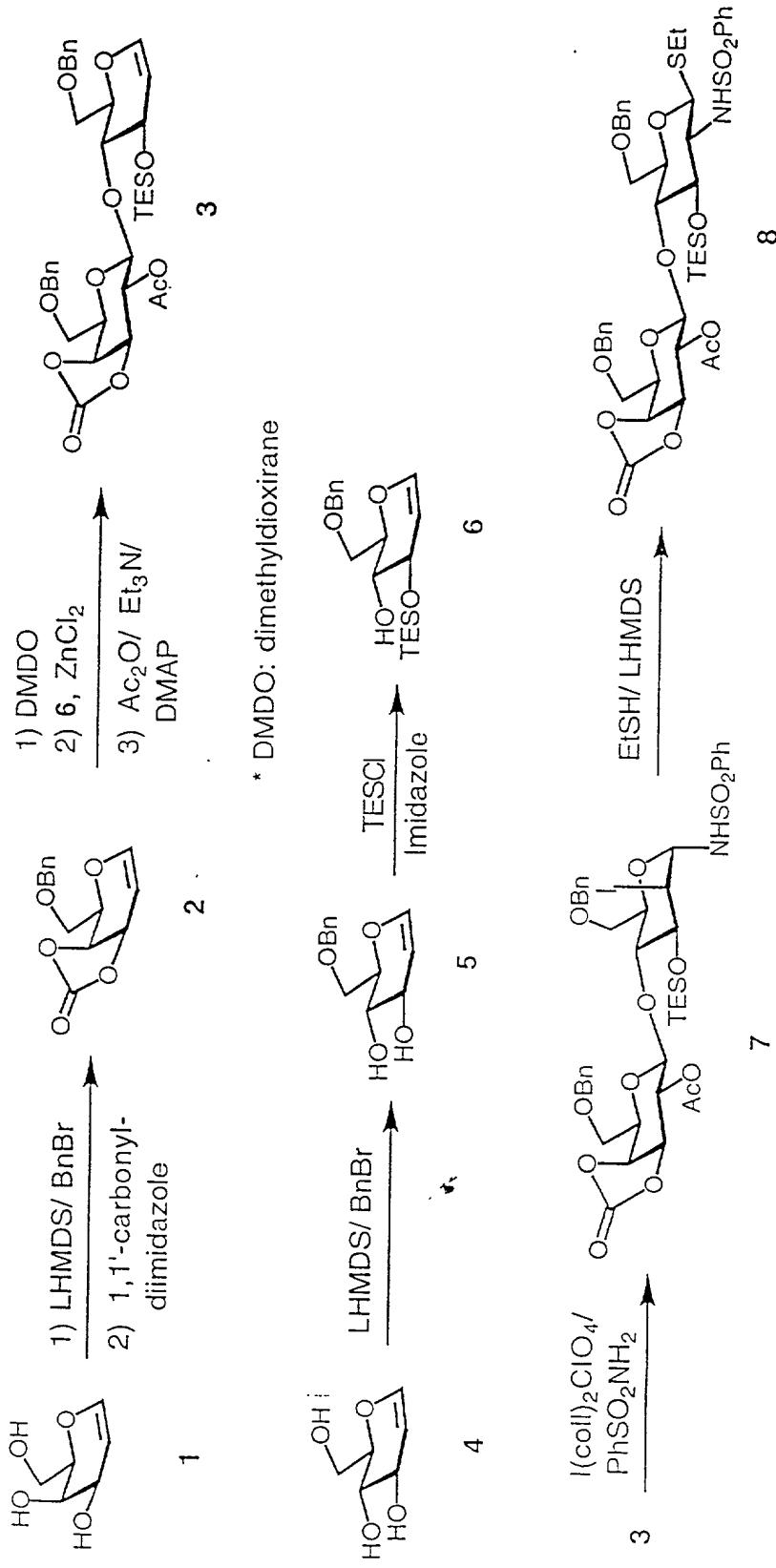
<sup>a</sup> Reagents: (a) 14,  $\text{Sn}(\text{OTf})_2$ ,  $\text{Tol}:\text{THF}(10:1)$ ,  $4 \text{ \AA MS}$  (60%); (b) (i) 3,3-dimethylidioxirane,  $\text{CH}_2\text{Cl}_2$ ; (ii)  $\text{EtSH}$ ,  $\text{CH}_2\text{Cl}_2$ ,  $\text{H}^+$  (cat); (iii)  $\text{Ac}_2\text{O}$ ,  $\text{Py}$ ,  $\text{CH}_2\text{Cl}_2$  60% (3 steps) (c) 17,  $\text{MeOTf}$ ,  $\text{Et}_2\text{O}:\text{CH}_2\text{Cl}_2$  (2:1),  $4 \text{ \AA MS}$  (55%); (d) (i) Lindlar's catalyst,  $\text{H}_2$ , palmitic anhydride,  $\text{EtIOAc}$ , 85%; (ii)  $\text{Na}, \text{NH}_3, \text{THF}$ ; (MeOH quench); (iii)  $\text{Ac}_2\text{O}$ ,  $\text{Et}_2\text{N}$ ,  $\text{DMAP}$ ,  $\text{CH}_2\text{Cl}_2$  (iv)  $\text{MeONa}, \text{MeOH}$ , 70% (3 steps); (e) (i)  $\text{Na}, \text{NH}_3, \text{THF}$ ; (MeOH quench); (ii)  $\text{Ac}_2\text{O}$ ,  $\text{Et}_3\text{N}$ ,  $\text{DMAP}$ ,  $\text{CH}_2\text{Cl}_2$ ; (iii) 3,3-dimethylidioxirane,  $\text{CH}_2\text{Cl}_2$ ; (iv) Allyl Alcohol (v)  $\text{MeONa}, \text{MeOH}$ , 60%..

Fig. 4



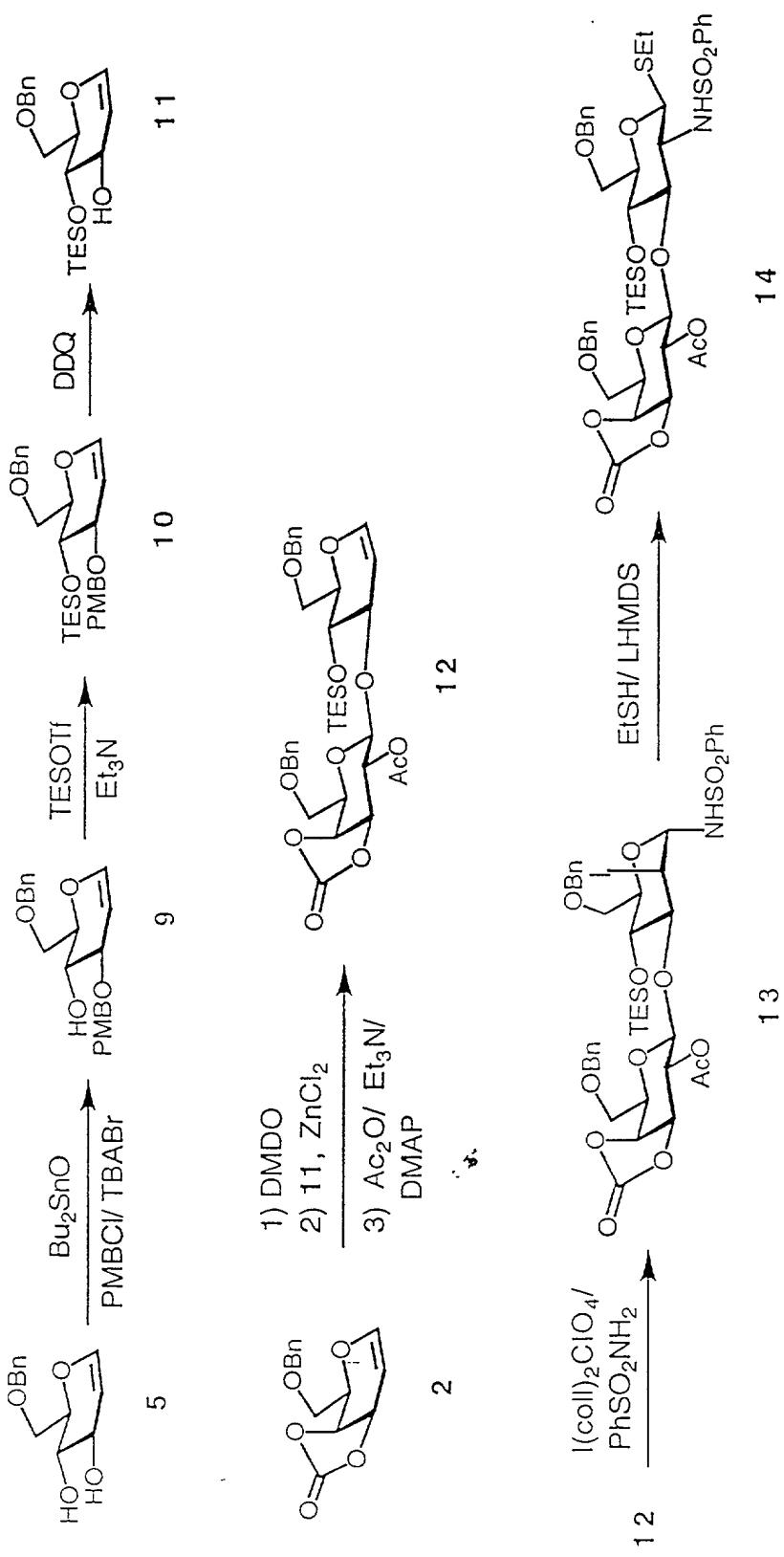
## Donor for Le<sup>X</sup> Part

Fig. 5



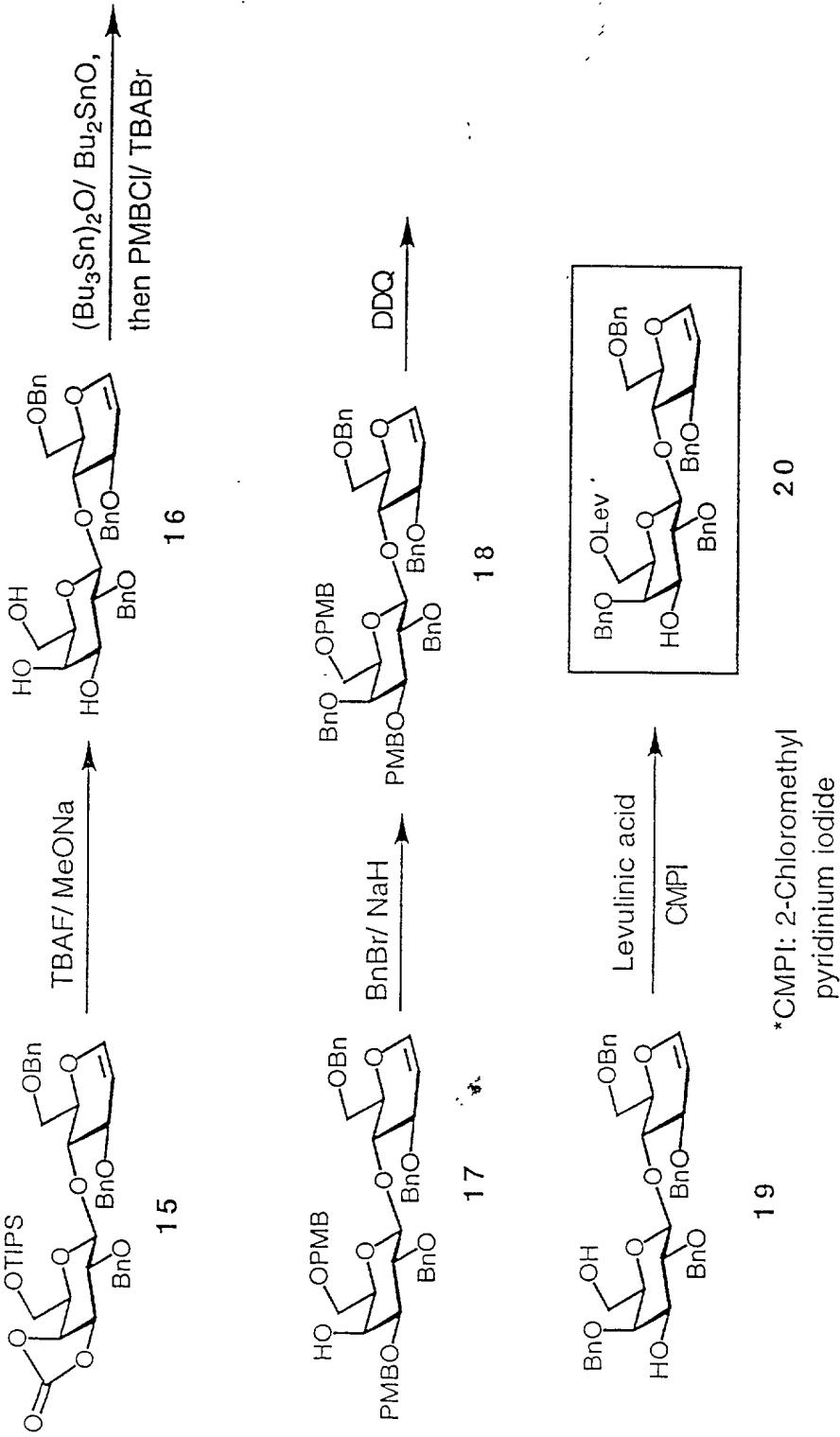
## Donor for Le<sup>a</sup> Part

*Fig. 6*



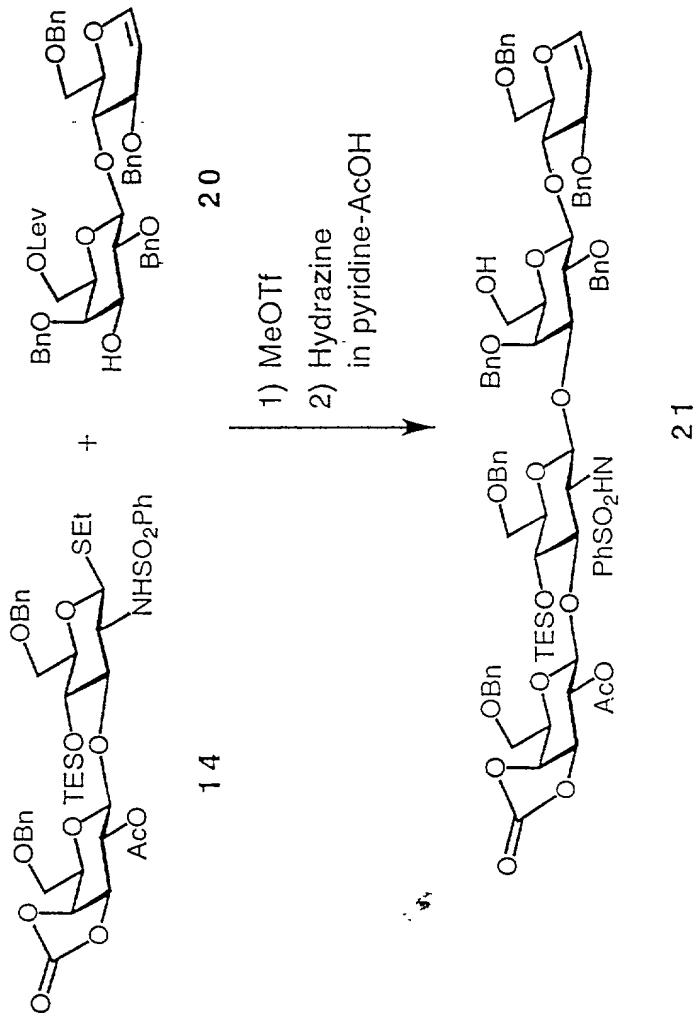
## Acceptor for N3 Antigens

Fig 7

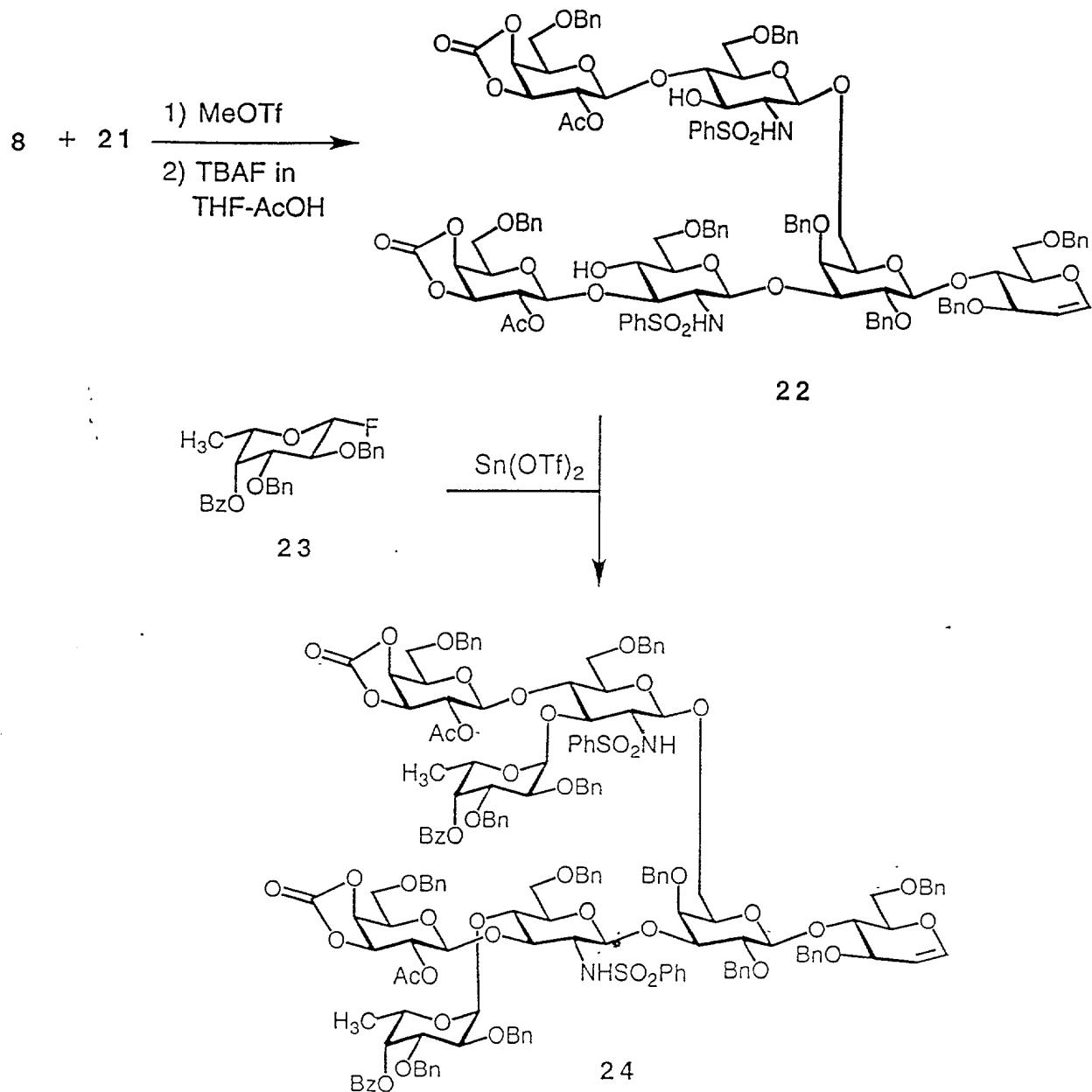


*Fig. 8*

## 2 + 2 Coupling for Major N3 Antigen

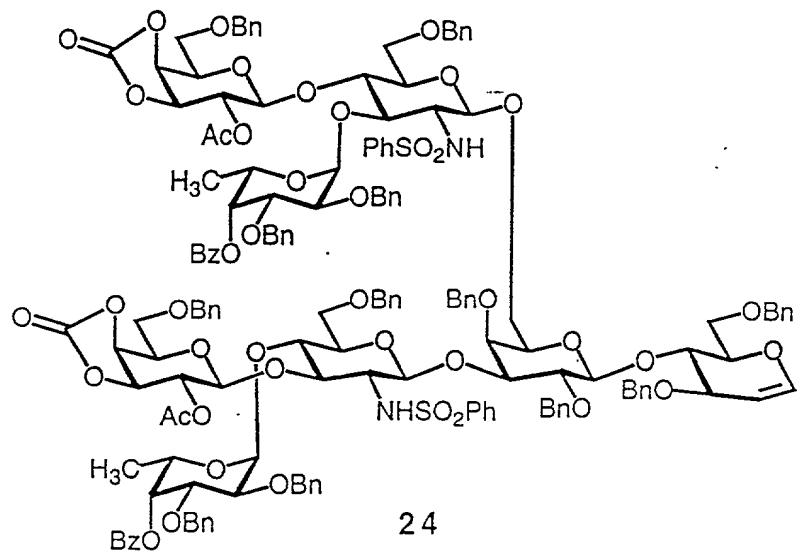


## 2 + 4 and +1,1 Coupling



↓  $\text{Sn(OTf)}_2$

## Deprotection for major-N3 Epitope



- 1)  $\text{Na}/ \text{NH}_3$ , then  
     $\text{Ac}_2\text{O}/ \text{Et}_3\text{N}/ \text{DMAP}$   
2) DMDO, then allyl alcohol  
3)  $\text{NaOMe}$

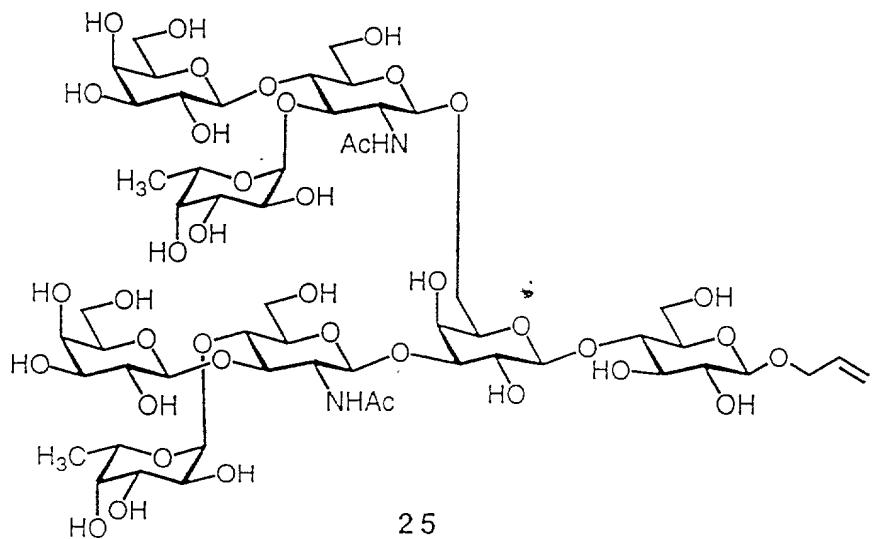
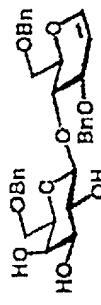
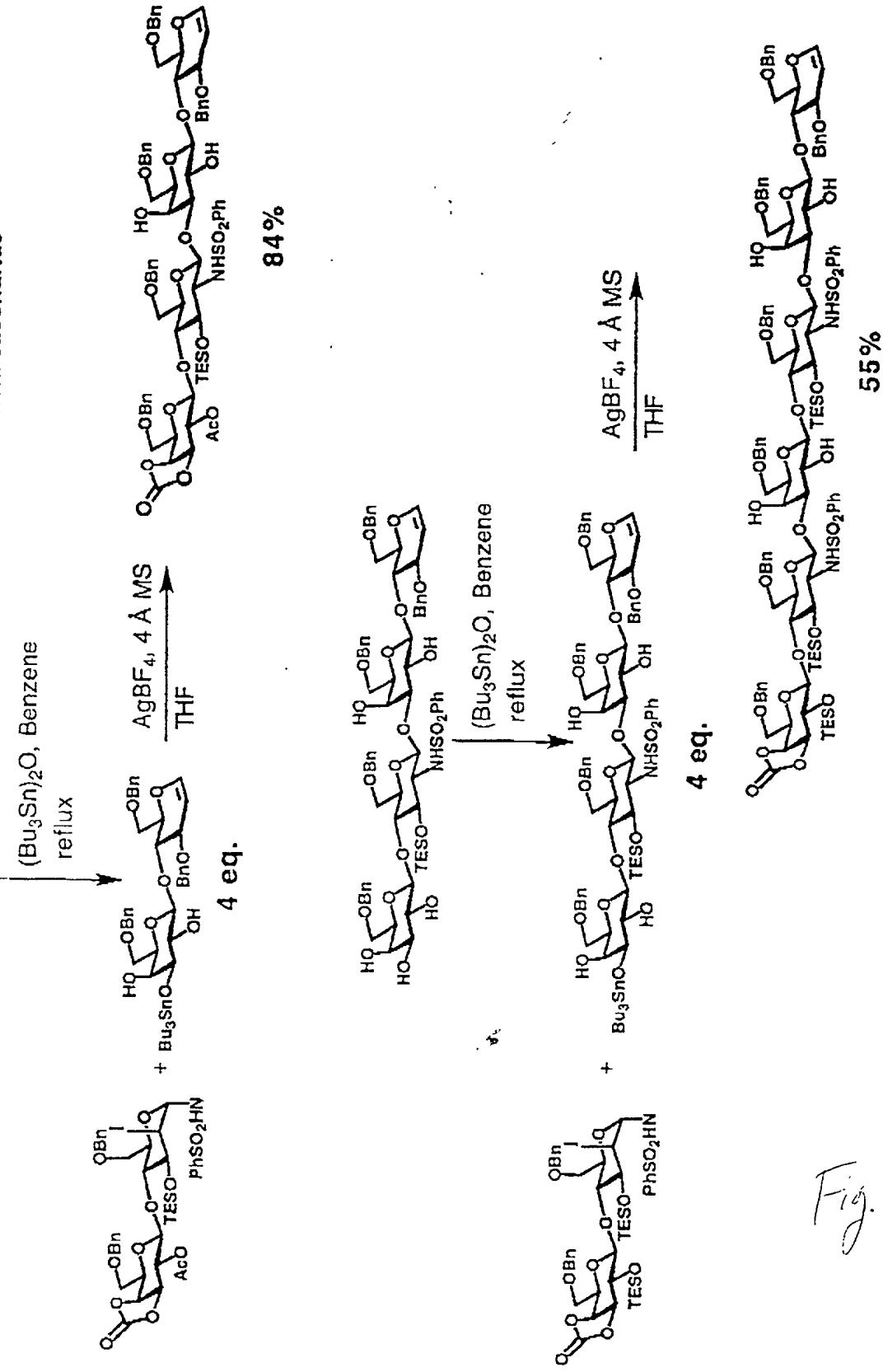


Fig 1c

W. H. Gish, Jr., Ph.D., 1970



### KH-1 Alternative Syntheses of tetra and hexasaccharide



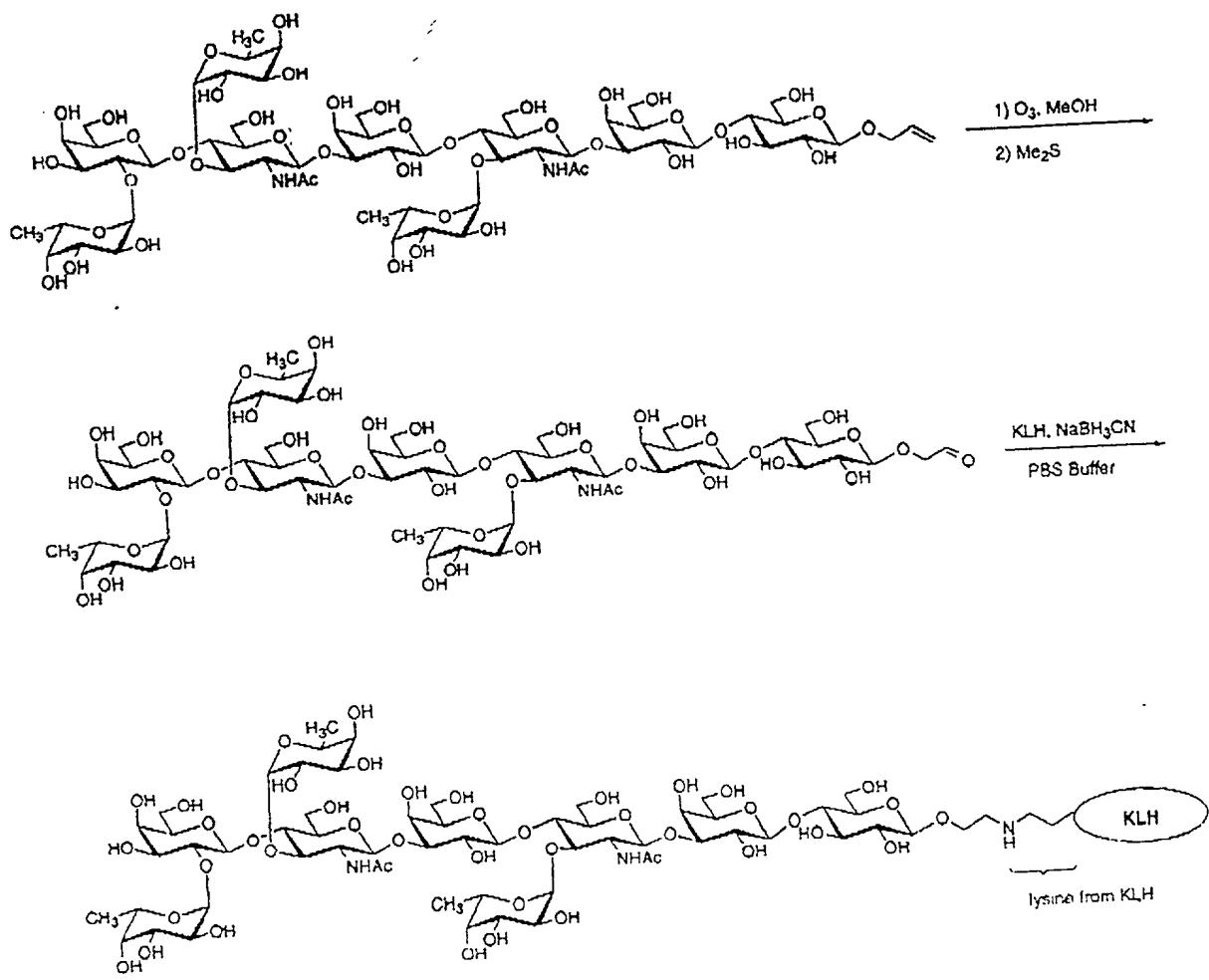
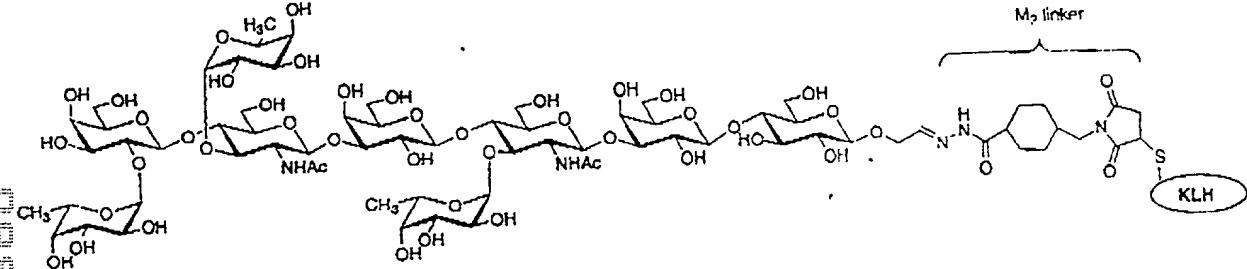
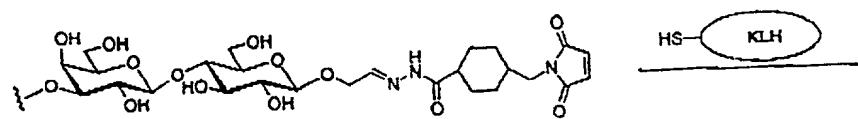
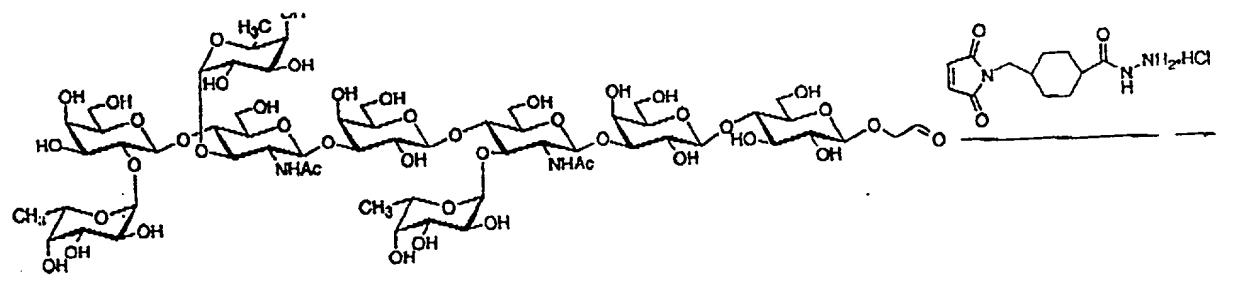


Fig. 12



Cross linker coupling of KH-1 to KLH

Fig 13